

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
28 June 2001 (28.06.2001)

PCT

(10) International Publication Number  
**WO 01/45639 A3**

(51) International Patent Classification<sup>7</sup>: C07H 21/04, A61K 48/00, 39/00, 39/07, 39/02, 39/385, C07K 14/00, 16/00, 17/00, A01N 63/00, C12N 15/63

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(21) International Application Number: PCT/US00/34912

(22) International Filing Date:  
21 December 2000 (21.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/171,459 22 December 1999 (22.12.1999) US

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(71) Applicants and

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(81) Designated States (national): AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

(88) Date of publication of the international search report:  
10 January 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR PROTECTING AGAINST LETHAL INFECTION WITH *BACILLUS ANTHRACIS*

(57) Abstract: Methods of inducing an immune response which protects a susceptible animal subject from lethal infection with *Bacillus anthracis* (*B. anthracis*) are provided. One method comprises administering an effective amount of wild-type, or preferably a mutated form of, *B. anthracis* lethal factor (LF) or an immunogenic fragment thereof to the subject. A second method comprises administering an effective amount of a mutated LF protein or an immunogenic fragment of an LF protein and an effective amount of the *B. anthracis* protective antigen (PA) or an immunogenic fragment of the PA protein to the subject. A third method comprises administering a polynucleotide or nucleic acid comprising a sequence encoding a mutated *B. anthracis* LF protein or an immunogenic fragment of an LF protein to the subject. A fourth method comprises administering a polynucleotide which comprises a coding sequence for a mutated LF protein or an immunogenic fragment of an LF protein and a polynucleotide which comprises a coding sequence for the *B. anthracis* PA protein or an immunogenic fragment thereof to the subject. The present invention also relates to a protein or peptide based-immunogenic composition for preparing a vaccine which is capable of prophylactically protecting a subject against lethal effects of infection with *B. anthracis* or exposure to a toxic agent which is produced by *B. anthracis*. The protein or peptide based immunogenic composition comprises a purified or recombinant LF protein or immunogenic fragment thereof and a purified or recombinant PA protein or immunogenic fragment thereof. The present invention also relates to a nucleic acid-based immunogenic composition comprising a nucleic acid which comprises a sequence encoding the LF protein or an immunogenic fragment thereof and a polynucleotide which comprises a sequence encoding the PA protein or an immunogenic fragment thereof.



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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/34912

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, E	COHEN et al. Attenuated nontoxinogenic and nonencapsulated recombinant Bacillus anthracis spore vaccines protect against anthrax. Infection and Immunity. August 2000, Vol. 68, No. 8, pages 4549-4558, especially pages 4549-4551, 4553-4557.	1-30
A	BARNARD et al. Vaccination against anthrax with attenuated recombinant strains of Bacillus anthracis that produce protective antigen. Infection and Immunity, February 1999, Vol. 67, No. 2, pages 562-567, especially pages 562, 564-567.	1-30
Y	US 5591631 (LEPLA ET AL) 07 JANUARY 1997 (07/01/97), see entire document, especially abstract, columns 3-6, Sequence Listing.	1-30

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search      Date of mailing of the international search report

03 MAY 2001

22 JUN 2001

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/34912

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5677274 (LEPPLA ET AL) 14 October 1997 (14/10/97), see entire document, especially abstract, columns 3-8, Sequence Listing, claims.	1-30
X ---	LITTLE et al. Comparative efficacy of Bacillus anthracis live spore vaccine and protective antigen vaccine against anthrax in the guinea pig. Infection and Immunity. May 1986, Vol. 52, No. 2, pages 509-512; especially abstract, pages 509, Table 2, page 510-511.	1, 2, 7, 8, 17, 18, 28 -----
Y		3-6, 9-16, 19-27, 29, 30
Y	BRAGG et al. Nucleotide sequence and analysis of the lethal factor gene (lef) from Bacillus anthracis. Gene. 1989, Vol. 81, page 45-54, especially, abstract, pages 46, 52, 53, figure 5.	1-30

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/34912**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US00/34912

A. CLASSIFICATION OF SUBJECT MATTER:  
IPC (7):

C07H 21/04; A61K 48/00, 39/00, 39/07, 39/02, 39/385; C07K 14/00, 16/00, 17/00; A01N 63/00; C12N 15/63

A. CLASSIFICATION OF SUBJECT MATTER:  
US CL :

514/2, 44; 530/350, 402, 395; 536/23.4, 23.1, 23.5, 23.6, 23.7; 435/320.1, 252.3; 424/184.1, 190.1, 93.1, 185.1, 197.11, 246.1, 93.46

B. FIELDS SEARCHED

Minimum documentation searched

Classification System: U.S.

514/2, 44; 530/350, 402, 395; 536/23.4, 23.1, 23.5, 23.6, 23.7; 435/320.1, 252.3; 424/184.1, 190.1, 93.1, 185.1, 197.11, 246.1, 93.46

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

MEDLINE, EMBASE, WPIDS, CABA, USPATFULL, GENBANK

inventor names, vaccine, immunogenic composition, protective antigen (PA), lethal factor (LF), recombinant protein, mutant protein, DNA vaccine, nucleic acid vaccine, polynucleotides, dna construct, B. anthracis, infection, anthrax

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-6 and 17-22, drawn to immunogenic composition and method of protecting an animal.

Group II, claims 7-16, drawn to method of protectin a susceptible animal.

Group III, claims 23-27, drawn to nucleic acid based immunogenic composition.

Group IV, claims 29-30, drawn to method of inducing an immune response comprising administering nucleic acids.

Group V, claims 28-30, drawn to method of inducing an immune response comprising administering proteins.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: because the special technical feature for each group is not the same. They require different methods or can be used in different ways other than that specifically claimed. The special technical feature of Group I is considered to be the specific method of protecting against disease using proteins. The special technical feature of Group II is considered to be the specific method of protecting against disease using the nucleic acids. The special technical feature of Group II is considered to be the nucleic acid sequences that encode the protein. The special technical feature of Group IV is considered to be the method of inducing an immune response using the nucleic acids. The special technical feature is considered to be the method of inducing an immune response using the protein.

Accordingly, Groups I-V are not so linked by the same of correspondingly special technical feature as to form a single general inventive concept.